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FIBRINOGEN RECOVERY IN TWO METHODS OF CRYOPRECIPITATE PREPARATION

RUTH DILLON

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MASTER OF SCIENCE

ABSTRACT

The purpose of this study was to determine if a relationship exists between the loss of fibrinogen and factor VIII and the volume of supernatant plasma removed during the preparation of cryoprecipitate from fresh frozen plasma. This was accomplished by measuring the fibrinogen and factor VIII content of aliquots of supernatant plasma collected at set points as it was removed. The aliquot collection points were percentages of the total volume of each unit. The amount of fibrinogen and factor VIII was then compared to the total amount of the constituents in the starting plasma and final cryoprecipitate.

Results indicated a significant difference between the methods in fibrinogen yield, factor VIII yield and final volume; the slow thaw method being superior to the quick thaw. An inverse linear relationship existed between fibrinogen loss and supernatant plasma volume in the quick thaw method, though the relationship is too small to be of practical value. No linear relationship was established between supernatant volume and the fibrinogen loss in the slow thaw method or factor VIII in either method. The model was able to account for all of the fibrinogen and factor VIII in the original starting plasma in the quick thaw method, but only 67% of the fibrinogen and 74% of the factor VIII in the slow thaw method.

FIBRINOGEN RECOVERY IN TWO METHODS OF CRYOPRECIPITATE PREPARATION

RUTH DILLON

Submitted to the Graduate College of Bowling Green State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 1989

Approved:

Advisor

Co-Advisor

ABSTRACT

The purpose of this study was to determine if a relationship exists between the loss of fibrinogen and factor VIII and the volume of supernatant plasma removed during the preparation of cryoprecipitate from fresh frozen plasma. This was accomplished by measuring the fibrinogen and factor VIII content of aliquots of supernatant plasma collected at set points as it was removed. The aliquot collection points were percentages of the total volume of each unit. The amount of fibrinogen and factor VIII was then compared to the total amount of the constituents in the starting plasma and final cryoprecipitate.

This study investigated the kinetics of fibrinogen and factor VIII loss in two methods of cryoprecipitate preparation, the quick thaw and slow thaw. Linear regression analysis was performed on the log transformation of the per cent of total fibrinogen and factor VIII in each aliquot against the per cent of total volume at which the aliquots were collected, to determine if a linear relationship exists. Mean, standard deviation and one way analysis of variance were also used to test the data generated in this study.

Results indicated a significant difference between the methods in fibrinogen yield, factor VIII yield and final volume; the slow thaw method being superior to the quick

thaw. An inverse linear relationship existed between fibrinogen loss and supernatant plasma volume in the quick thaw method, though the relationship is too small to be of practical value. No linear relationship was established between supernatant volume and the fibrinogen loss in the slow thaw method or factor VIII in either method. The model was able to account for all of the fibrinogen and factor VIII in the original starting plasma in the quick thaw method, but only 67% of the fibrinogen and 74% of the factor VIII in the slow thaw method.

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INTRODUCTION AND REVIEW OF LITERATURE

Hemostasis

The body's mechanism to stop bleeding is called hemostasis. Hemostasis is an on-going, dynamic process involving the interaction of the vasculature, platelets and circulating plasma proteins known as coagulation or clotting factors.[1,2,3] Hemostasis can be thought of as occurring in two stages, primary and secondary.[1] Primary hemostasis is plugging the hole in the vessel to prevent blood from flowing out and involves the vasculature and platelets. Secondary hemostasis is cementing the platelet plug in place. Fibrin, a cleavage product of the coagulation protein fibrinogen, is the cement.

Coagulation, the process of fibrin clot formation, is a complex series of reactions involving plasma proteins, known as the coagulation factors, calcium ions and phospholipids, that occur during secondary hemostasis.[1,2] The coagulation factors are listed in Table 1. Coagulation factors are designated by Roman numerals in addition to a common name and an "a" subscript denotes the active form of the factors.[4] The coagulation proteins are produced in the liver.[4,5] The majority of the proteins (factors II, VII,IX, X, XI, XII and Fletcher factor) are serine proteases which circulate in the plasma in an inactive form until needed at

TABLE 1: COAGULATION FACTORS AND NOMENCLATURE

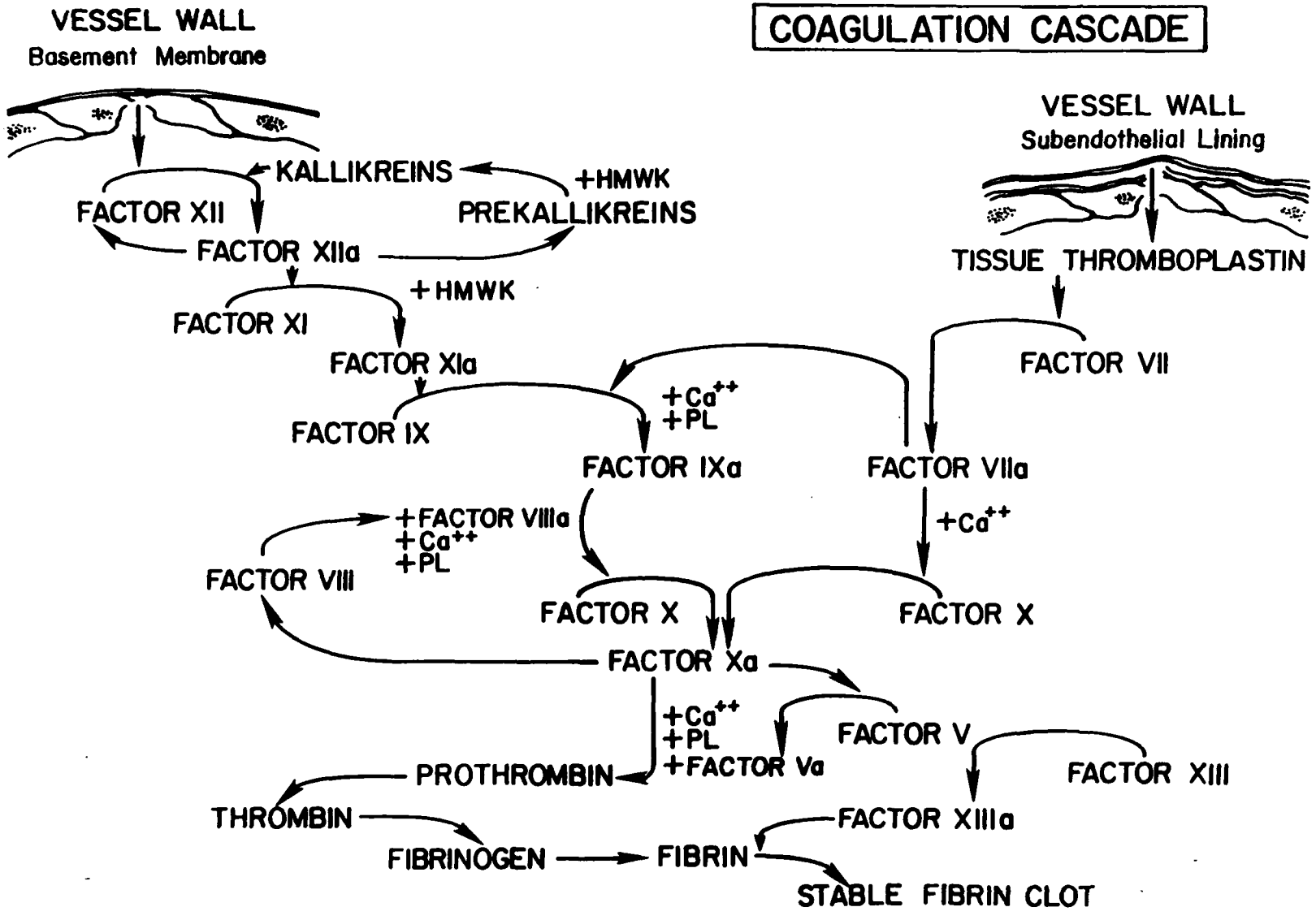
Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin
V	Labile factor, proaccelerin
VII	Stable factor, proconvertin
VIII	Antihemophilic factor (AH)
IX	Christmas factor
X	Stuart Prower factor
XI	Plasma thromboplastin antecedent
XII	Hageman factor, Contact factor
---	Prekallikrein, Fletcher factor
---	High Molecular Weight Kininogen, Fitzgerald factor

a site of hemorrhage. Factor XIII is also a protease, however, it is a transglutaminase. Serine proteases are enzymes whose active sites are serine residues, while transglutaminases are enzymes that catalyze the peptide bond formation between amino acids, specifically amine groups of lysine and l groups of glutamine. Other coagulation protein factors (V, VIII) and calcium ions serve as cofactors to the proteases and accelerate or enhance the reactions. Phospholipids, on the other hand, are not activated nor consumed, but provide a surface on which some of the reactions can occur. High molecular weight kininogens (HMWK) act similarly to phospholipids by providing a surface for early activation reactions. The final substrate is fibrinogen (factor I), which is not a serine protease, but a dimeric protein.

The reactions of the coagulation system occur sequentially, in a cascade fashion (Figure 1).[6,7] The reactions involve conversion of the inactive coagulation factors to their active form. The substrate of this active form is another coagulation factor, which then becomes activated, hence the term cascade. Another characteristic of the coagulation scheme is its ability to amplify itself thousands of times with each reaction. This amplification occurs as a result of an enzyme molecule's ability to act upon numerous molecules of substrate. An example given by McFarlane,[7] is the activation of one molecule of enzyme, activated as a result of an initial reaction may activate

FIGURE 1: Figure 1 depicts the series of reactions that occur during the process of coagulation. The process is started by damage to the vessel wall which activates factor XII by contact with basement membrane and factor VII by release of tissue thromboplastin. Each step in the cascade involves the conversion of an inactive protein procoagulant to its activated protease form. The activated factor in turn acts upon the next procoagulant in a "cascade" fashion. Some steps require cofactors such as calcium (Ca^{++}), phospholipids (PL) and factors V and VIII. The central reaction of the cascade is thrombin (IIa) generation. The final product is fibrin which results from the proteolytic cleavage of the dimeric protein fibrinogen by thrombin.

COAGULATION CASCADE



ten molecules of substrate itself, resulting in $1x10^6$ molecules of the final product, fibrin. Additionally, the cascade has numerous positive feedback loops which further amplify the reactions.

The central reaction of the coagulation system is the activation of prothrombin (factor II) to thrombin (IIa).[8] The proteolytic activity of thrombin cleaves the fibrinogen molecule into fibrin monomers which polymerize to form the fibrin clot. Conversion of prothrombin to thrombin can be initiated as a result of two principal routes or "pathways", the intrinsic and extrinsic.[8] The pathways differ primarily in the mode of initiation and the factors involved. The extrinsic pathway is initiated through contact activation and complex formation of tissue thromboplastin, which is released from damaged subendothelial tissue, and factor VII (proconvertin) which circulates in the blood. The factor VIIa/tissue thromboplastin complex then activates factor X (Stuart Prower factor) in the presence of calcium ions.[8] Activated factor X enzymatically alters prothrombin (factor II) to the active form thrombin (IIa).[9] This reaction is greatly accelerated by activated factor V (Va) in the presence of calcium ions and platelets.[10,11] Factor V is itself activated by Xa and thrombin in a positive feedback loop providing acceleration.[10,11]

The intrinsic pathway begins with the activation of factor XII when it binds with the negatively charged surface

of the exposed basement membrane and collagen.[12,13] Activated factor XII (XIIa) has several substrates: prekallikrein, factor XI, and other factor XII molecules.[13,14] High molecular weight kininogens also play a role by complexing with the substrates, factor XI and prekallikreins, to facilitate the action of XIIa.[14,15] Prekallikreins are converted to kallikreins by XIIa, which in turn convert more factor XII to XIIa, another positive feedback loop, which further accelerates the reactions.[13] Factor XI is converted to its activated form, XIa, by XIIa.[13] Activated factor XI, in the presence of platelet factors and calcium ions, will in turn activate factor IX (Christmas factor).[16] Additionally, it has been demonstrated that factor IX is also activated via the extrinsic pathway by factor VIIa/thromboplastin complex, still another example of amplification within of the coagulation cascade.[17] Activated factor IX, activates factor X,[18] a reaction which occurs very slowly without the interaction of required cofactors, activated factor VIII (VIIIa), platelet phospholipids and calcium ions.[11,19] Factor IXa complexes with these cofactors, rapidly completing the reaction.[11,18] As in the intrinsic pathway, activated factor X cleaves prothrombin to form thrombin.

Fibrin monomers, formed through the proteolytic activity of thrombin on fibrinogen, align and polymerize to form a loose mesh network.[20] This fibrin polymer is stabilized and

strengthened by activated factor XIII (XIIIa) as it forms covalent crosslinkages among the fibrin monomers.[20]

Cryoprecipitate

Cryoprecipitate is the cold insoluble protein precipitate that results when fresh frozen plasma is thawed at 4°C. After thawing, most of the supernatant plasma is removed leaving the protein precipitate rich in factor VIII, von Willebrand factor, fibrinogen, factor XIII and fibronectin. The resulting product contains approximately 25-50% of factor VIII and fibrinogen, 40-70% von Willebrand factor and 20-30% of the factor XIII of the original plasma in a volume of 10-25 milliliters (ml).[21,22] The product is refrozen and stored at -18°C for up to a year from the date of collection of the original unit of blood.[23-25] Because of its low volume of isoagglutinins and absence of red blood cells, cryoprecipitate can be transfused without regard for the ABO group or Rh type of the original unit, though ABO compatibility is preferred.[24]

Cryoprecipitate was developed by Pool and Shannon as a source of concentrated factor VIII for the treatment of hemophilia A.[26] Prior to the development of cryoprecipitate, severe hemophilia A was a debilitating disease, leaving the victims that survived to adulthood disabled due to chronic bleeding into the joints.[21,27] As

stated previously, cryoprecipitate has approximately 25-50% of the factor VIII of the starting plasma, yet only one-fifteenth the plasma volume. Cryoprecipitate revolutionized the treatment of hemophilia by providing a low-volume concentrated source of factor VIII, eliminating problems of volume overload. It has, however, been largely replaced by high-purity, concentrated lyophilized preparations of factor VIII which became available in the 1970s.[28,29]

Volumes of research have been reported on the factor VIII content of cryoprecipitate and the variables influencing the factor VIII yield. A recurring observation is the variability in yield of factor VIII (as well as fibrinogen) in cryoprecipitate.[27,30-38] Many factors have been found to influence the yield including: variable levels of factor VIII in the individual donors,[31] adequate mixing of blood during collection,[32] amount of storage time of blood prior to processing,[30,32] starting plasma volume,[33,34] age of starting plasma,[34,35] and the rate of freezing plasma.[34,35,36] These data were used as a basis in establishing a minimum standard of 80 units of factor VIII per unit of cryoprecipitate by the American Association of Blood Banks (AABB)[23] and the US Department of Health and Human Services, Food and Drug Administration (FDA).[25] However, no such requirements have been established for the fibrinogen content in cryoprecipitate.

Factor VIII

As stated previously, factor VIII is a coagulation protein that serves as a cofactor, along with phospholipids and calcium ions, that accelerate the activation of factor X by factor IXa.[11,19] It circulates in the blood non-covalently bound to von Willebrand factor, another protein that is essential to platelet adhesion.[19] These are two distinct proteins, made by different genes with different biological functions and immunological properties.[19] Factor VIII refers to the protein that corrects the clotting defect of hemophilic plasma (its function designated factor VIII:C for factor VIII clotting protein), while vWf refers to the adhesive protein von Willebrand factor.[19,39] Immunologically, the proteins can be differentiated and quantitated using antibodies formed against the separate antigenic structures of the two molecules. The factor VIII antihemophilic activity when measured in this fashioned is designated factor VIII:CAg, while the von Willebrand antigen is designated factor VIII-related protein (factor VIII:RP).[19]

The structure of factor VIII long eluded scientists because of; 1) the difficulty in obtaining pure factor VIII free from contaminating vWf, 2) its low plasma concentration and 3) its susceptibility to enzyme degradation.[11] The first great stride to unlocking the

mysteries of the chemical nature and molecular composition of factor VIII came when bovine,[40] porcine[41,42] and finally human factor VIII[43] fragments were purified to sufficient homogeneity to allow amino acid sequencing in the early 1980s. This allowed two independent research groups to obtain protein segments, and from those segments to isolate the factor VIII gene on the X chromosome.[44-47] The factor VIII gene codes for a protein of 2332 amino acids with a calculated molecular weight of Mr 264,763.[44,45,46] The factor VIII molecule has three major domains of homology designated A, B and C.[11,44,46,47] The A domain is repeated three times in each molecule, the C domain twice, while the B domain occurs only once in each factor VIII molecule. These domains occur in the order A_1, A_2, B, A_3, C_1, C_2 as depicted in Figure 2. It is near the junctions of these domains that enzyme cleavage occurs.[11]

Native factor VIII is a large glycoprotein molecule with a reported molecular weight of 270,000-330,000 based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).[19] This molecular weight closely approximates the calculated molecular weight when glycosylation of all potential n-linked oligosaccharide sites has occurred.[11]

Factor VIII is extremely sensitive to enzyme degradation, specifically by thrombin and factor Xa, both in vivo and in the purification process.[11,19,44,48] As a result of this, several smaller protein fragments are

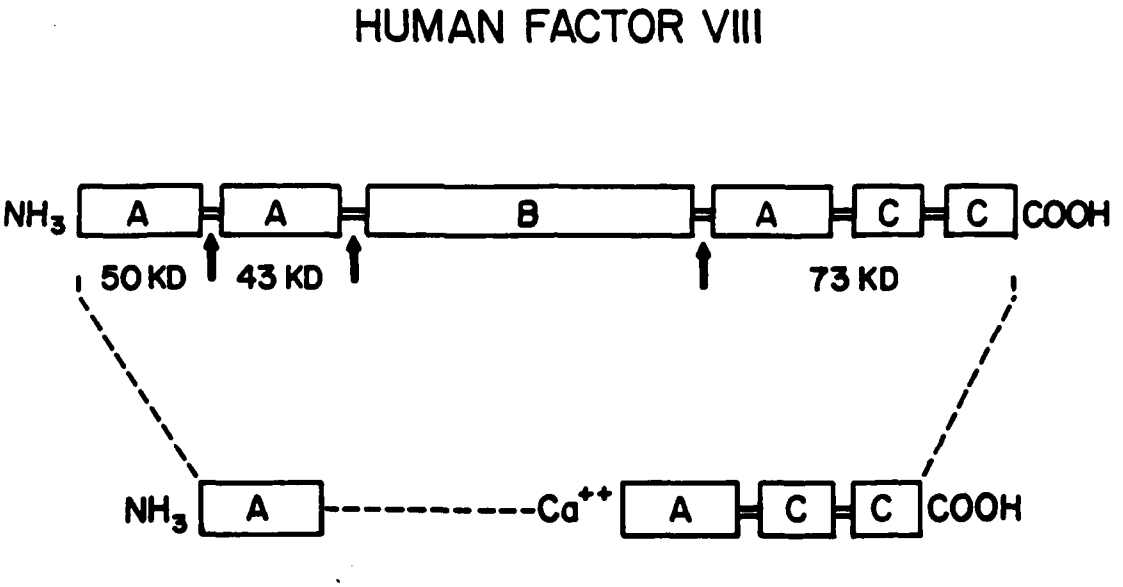


FIGURE 2: The factor VIII molecule is a large glycoprotein molecule (MW≈330KD) with the three major domains of homology designated A, B and C. The A domain occurs three times in the molecule, the first two originating at the amino terminus, and the third separated by a single B domain. The C domain is repeated twice and follows the third A domain at the 1 terminus. The activated factor VIII molecule consists of calcium (Ca^{++}) crosslinked fragments which occur after enzymatic excision of the 1 terminal A domain and the single B domain.

created. These fragments are divided into two broad classes based on molecular weight; one class with an initial molecular weight of 90,000-210,000 and a second class initially of molecular weight 80,000.[11,19,44,48] These two fragment classes originate from the amino and carboxyl termini of the native factor VIII molecule respectively.[44] The 210,000 fragments are further degraded into still smaller 90,000 fragments, while the 80,000 fragments are cleaved to 73,000 fragments.[11,19,44,48] These fragments become crosslinked by calcium ions to form activated factor VIII. Inactivation of factor VIII involves still further proteolytic degradation of the 90,000 and 73,000 fragments.[11,19,44,48]

The disease process associated with deficient factor VIII activity is hemophilia A. Hemophilia A is a sex-linked disease characterized by bleeding disorders due to low levels of functional factor VIII.[49,50] Owing to the factor VIII gene's location on the X chromosome, hemophilia A is, with rare exception, a disease of males. Females, on the other hand, may be carriers of the disease, but do not suffer from the disease because of the presence of a second non-defective gene.[49,50]

The incidence of hemophilia A has been reported at 5-20 cases per 100,000, the highest for all inherited coagulation disorders.[49,50] The frequency and severity of hemorrhagic episodes in hemophilias is directly related to

the factor VIII:C activity. Severe hemophiliacs are those whose factor VIII:C levels are less than 1% of normal, mild hemophiliacs greater than 5%, and moderate hemophiliacs fall in between.[49] Severe hemophiliacs have frequent recurrent hemorrhages and may require repeated therapeutic intervention, while mild hemophiliacs generally only have bleeding episodes following trauma or surgical procedures.[49] As would be expected, moderate hemophiliacs fall in the middle of the two extremes and the clinical pictures vary.[49]

Fibrinogen

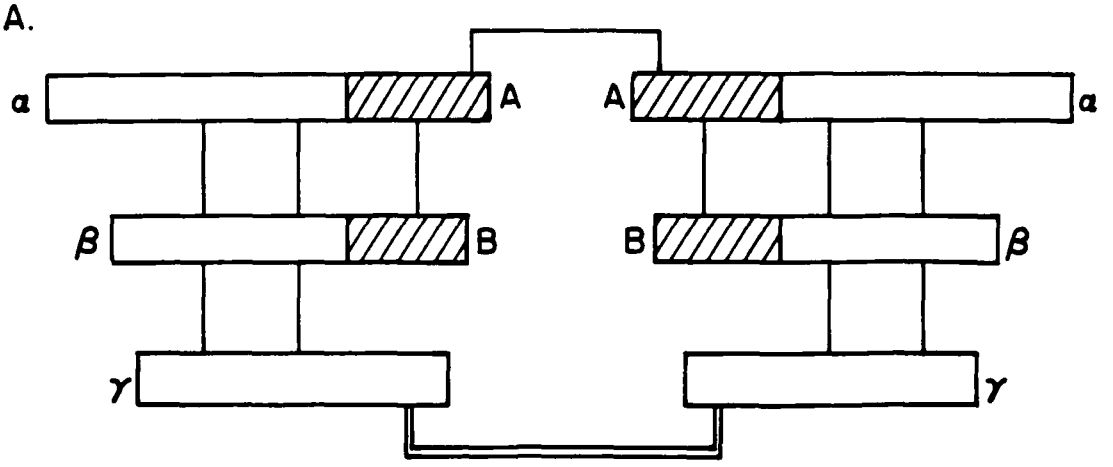
The structure of the fibrinogen molecule was first described by Hall and Slate in 1959,[51] and further developed by Tooney and Cohen in 1977,[52,53] Doolittle et al. in 1978,[54] and Weisel in 1985.[55] Fibrinogen is a dimeric glycoprotein composed of three pairs of polypeptide chains linked by disulfide bonds (Figure 3).[56] The polypeptide chains are designated A α, B β, and τ. Each fibrinogen molecule contains two identical sets of these chains. The A and B portion of the α and β chains respectively, are fibrinopeptides cleaved from the intact molecule by thrombin. Fibrinopeptide A is cleaved first, followed by a lag phase and cleavage of fibrinopeptide B.[56,59] The resulting fibrin monomers then polymerize end-to-end and laterally forming a soluble fibrin mesh. Activated factor

FIGURE 3A. Figure 3A diagrammatically depicts the fibrinogen molecule. The molecule consists of three pairs of polypeptide chains named A α, B β and γ linked by disulfide bonds represented by the vertical lines in the diagram. The A and B portions of the chains (hashed area of chains) are fibrinopeptides which are cleaved by thrombin during the coagulation process to form the fibrin clot.

FIGURE 3B. Figure 3B is a three dimensional representation of fibrinogen structure as detailed by Weisel.[55] The three chains are intertwined in an α helical coiled-coil with the amino (NH_3) termini forming a central zone in the molecule. The carboxyl (COOH) ends of the chains extend outward; the B β and γ chains (white and black chains in diagram) form two zones at the outer edges of the molecule, while the A α (stippled chain) folds back to form a carboxyl zone near the central amino zone.

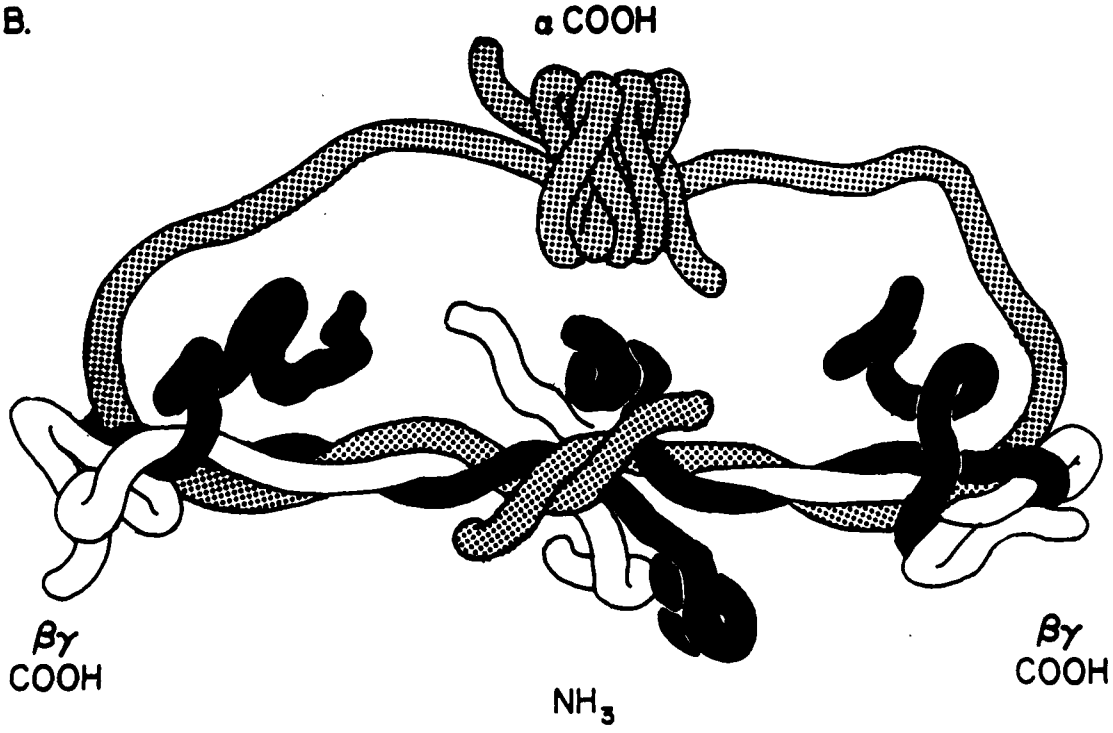
STRUCTURE OF HUMAN FIBRINOGEN

A.



COOH NH_3 COOH

B.



XIII then covalently crosslinks these monomers to form a non-soluble fibrin clot.[20,49]

The complete amino acid sequence of the fibrinogen molecule was reported in 1978 by Doolittle.[54] Each fibrinogen molecule has a total of 1482 amino acid residues; each γ chain having 411, each B β chain 461 and each A α chain having 610.[57] The six polypeptide chains are arranged with the amino terminals of each chain clustered in a central zone, linked by disulfide bonds. The A and B fibrinopeptide portions of the α and β chains are located in this central amino domain. The peptide chains extend outward from the central domain intertwining into an alpha helical "coiled-coil" with the carboxyl termini of the β and γ chains lying at the ends of the helical coils. These carboxyl ends form separate domains. The carboxyl termini of the α chains, on the other hand, extend beyond the γ-β carboxyl domains and folds back into a separate α carboxyl domain near the central amino center.[55,57]

The fibrinogen molecule is a large glycoprotein with a molecular weight of 340,000. The polypeptide chains have molecular weights of 64,000, 75,000 and 48,000 for the A α, B β, and γ chains, respectively.[56] Fibrinogen is synthesized in the liver at a rate of 1.7-5.0 g/day.[58] The mean plasma concentration of fibrinogen has been reported at 280 mg/dl (range 165-485 mg/dl)[59] and 284 mg/dl (range 213-355 mg/dl).[60] Fibrinogen belongs to the family of acute

phase reactants, the concentrations for which can increase twice normal in response to stress, such as inflammation, surgery or increased consumption.[61] The half life of fibrinogen is reported by Collen et al. to be 3-5 days.[60]

Disorders of fibrinogen can lead to hemorrhagic problems and may result from a variety of disorders. These disorders are generally classed into two broad categories; abnormal structure or decreased quantity.[62] The dysfibrinogenemias, qualitative variations of the fibrinogen molecule, are most often congenital in nature due to amino acid substitutions causing either dysfunctional fibrinopeptide release or fibrin polymerization.[57,62,63] Dysfibrinogenemia can occur in severe liver disease as well.[62] The clinical manifestations vary widely from few bleeding problems to frequent hemorrhagic events, as well as excess clotting resulting in thromboembolytic episodes (blockage of vessels by clots).[62,63] The severity and frequency of bleeding episodes is dependent upon the level of fibrinogen.[63] It has been demonstrated that patients with at least 100 mg/dl of fibrinogen generally don't have bleeding problems, while patients with less than 100 mg/dl tend to have bleeding complications.[62]

Afibrinogenemia, a complete absence of fibrinogen, is a very rare congenital disorder inherited in an autosomal dominant fashion.[64] Patients with afibrinogenemia often have bleeding tendencies and hemorrhagic episodes,

particularly during the neonatal and childhood period.[64]

Hypofibrinogenemia, reduced levels of fibrinogen, is far more frequent and can be due to decreased production or increased consumption. Decreased production, when congenital, is inherited in an autosomal fashion.[64] More often, however, decreased fibrinogen production is acquired, most frequently secondary to liver disease, particularly cirrhosis.[59,62] The decrease is generally moderate, but can be severe with bleeding problems generally occurring when the fibrinogen level falls below 100 mg/dl.[62]

One of the most common causes of increased consumption of fibrinogen is Disseminated Intravascular Coagulation (DIC). DIC is characterized by widespread coagulation and simultaneous fibrinolysis.[65] DIC can be triggered by a variety of pathological processes including placental abruptio (premature separation of the placenta from the uterine wall), retention of a dead fetus, amniotic fluid leakage into maternal circulation, gram-negative septicemia, trauma or surgical complications.[62,63,65,66] DIC results in consumption of platelets and those coagulation factors that are consumed during the clotting process such as fibrinogen.[65,66]

Products for Treatment of Fibrinogen Disorders

The treatment of fibrinogen disorders varies with the clinical state of the patient. Patients who have no bleeding diathesis generally require no supplemental treatment. On the other hand, those patients with hemorrhagic episodes require medical intervention and support. Treatment for these patients is replacement therapy, supplying fibrinogen through transfusion.

The first treatment for fibrinogen disorders, as well as all other coagulation disorders, was plasma.[21] This therapy was often times complicated by circulatory overload due to the large amount of plasma that was needed to correct the deficiency. In 1946, Cohn and coworkers developed a method to fractionate various proteins from plasma based upon the different solubilities in a low ionic strength medium when the pH and alcohol content of the medium was altered at low temperatures.[67] A product of fraction I of the Cohn procedure was a dried plasma, rich in fibrinogen.[68,28] This fibrinogen concentrate, first marketed in 1947 under the name Fibrinogen (human), overcame the fluid overload problems incurred when normal plasma was used.[68] This fibrinogen concentrate also contained substantial quantities of factor VIII and other plasma proteins.[69] Because of this, it was also one of the first concentrates used to treat Hemophilia A, as well as

fibrinogen deficiencies.[69]

Fibrinogen (human) treatment was not without significant risk, the most critical being the high rate of viral hepatitis transmission; rates of 1.7-55% were reported.[63,68,70-72] The high rate of hepatitis transmission was attributable to the large numbers of donors included in each plasma pool, from 2000-20,000,[63,73] and the inability of fibrinogen to withstand heat treatment necessary to inactivate the hepatitis virus.[63,70] In 1978, Fibrinogen (human) concentrate was removed from the commercial market by the FDA.[74]

Following the termination of fibrinogen concentrate production in 1978, the treatment of choice for fibrinogen disorders became cryoprecipitate.[21,22,63,75,76] Cryoprecipitate has the advantage of being produced from single donors, therefore significantly reducing the donor exposure and resultant risk of viral disease transmission.[21,28,29,76]

Fibrinogen in Cryoprecipitate

With the advent of commercial fractionated factor VIII concentrates in the 1970s, the research in cryoprecipitate began to focus on fibrinogen and the other constituents of cryoprecipitate. In Pool and Shannon's initial report, they noted that it contained an appreciable amount of fibrinogen.[26] At this time commercial fibrinogen

concentrate was still available so no therapeutic use of this information was made. As more and more evidence began to link the fibrinogen concentrate with hepatitis transmission, cryoprecipitate became an alternative for fibrinogen therapy as previously noted. One of the earliest reports of fibrinogen content in cryoprecipitate was presented by Oka et al. in 1968 who reported a yield 285-300 mg/unit (n=10).[76] Soloway and Bereznak determined the fibrinogen concentration of cryoprecipitate not by assaying the cryoprecipitate, but by monitoring the fibrinogen levels in two hemophiliac patients receiving cryoprecipitate for factor VIII therapy.[77] They determined the mean fibrinogen concentration of cryoprecipitate to be 250±30 mg/unit, with a range of 75-457 mg/unit. These data have long been the accepted reference for fibrinogen content of cryoprecipitate.[22] Recent studies have confirmed this work, reporting means of 139-266 mg/unit.[75,78,79] Like factor VIII, fibrinogen content in cryoprecipitate shows great variability.

In their comprehensive report on initial experiences with cryoprecipitate, Oka et al. also studied recovery of fibrinogen in the supernatant plasma removed from 17 units of cryoprecipitate.[76] They reported a median fibrinogen content in the supernatant plasma of 110 mg/100 ml (range 50-160 mg/100 ml) compared to a median of 220 mg/100 ml (range 180-250 mg/100 ml) in the original starting plasma, a

recovery of 50%. This was in contrast to a recovery of only 12-28% of factor VIII in the supernatant. Prowse and McGill[78] in 1979 evaluated a newer method of cryoprecipitate production, the 'Mason' continuous-thaw-siphon technique, versus two established methodologies, quick-thaw (2 hours, 4°C circulating water bath) and slow-thaw (4°C refrigerator overnight). As part of this study, fibrinogen was assayed in addition to factor VIII. They reported a mean of 225 mg fibrinogen per unit of cryoprecipitate by the Mason method (n=45, volume = 32±2.2 ml), 155 mg for the quick-thaw method (n=25, volume = 36±4.2 ml), and 139 mg for the slow-thaw method (n=25, volume = 36±4.1 ml). The average total fibrinogen content for the starting plasma was 492 mg, yielding 46%, 32% and 28% recovery of the starting fibrinogen for the methods, respectively. The factor VIII activity in the different methods evaluated in this study was similar to that of fibrinogen, 175 (71% recovery), 125 (53%) and 68 (28%), respectively, for each method. Despite the apparent superiority of the Mason thaw-siphon technique, this method never achieved wide use because it is labor intensive and too cumbersome for large scale production.[38]

The removal of fibrinogen concentrate from the commercial market prompted Ness and Perkins to study the extent of fibrinogen variation in cryoprecipitate.[75] They assayed the fibrinogen content of 88 units of cryoprecipitate prepared by five different blood banks.

They reported a mean fibrinogen of 266.4 mg/unit, with a range of 63-417 mg/unit. The mean starting plasma volumes varied between the five blood banks preparing the cryoprecipitate; means of 210 to 250 ml. The volumes of cryoprecipitate likewise varied, from 10-25 ml/unit.

In the most recent studies of fibrinogen yields in cryoprecipitate, Hoffman et al. studied the effect of reducing the final volume on the fibrinogen recovery.[79] They studied 23 units of "low-volume" cryoprecipitate (mean volume 4.0±1.6 ml, range 1.0-7.1 ml) and 8 "regular-volume" cryoprecipitate units (mean volume 15.6±2.1 ml, range 14-19 ml). The mean starting plasma volume was 217±19 ml and 207±14 ml, respectively, for the two products. The fibrinogen for the "low-volume" cryoprecipitate was 100 mg/unit, while that of the "regular-volume" units was 142 mg/unit, a difference of 42 mg/unit. While the difference was not statistically significant, it did suggest a correlation between fibrinogen yield and residual plasma volume. In a follow-up report, Hoffman studied the fibrinogen content of cryoprecipitate produced by three methods; quick-thaw low volume, quick-thaw regular volume, and overnight thaw regular volume.[80] The mean cryoprecipitate volumes of the three methods were 8.5±1.7 ml (n=11), 15.9±3.5 ml (n=16) and 14.2±2.1 ml (n=20) respectively. The fibrinogen content of the cryoprecipitate prepared by the three methods was 191±37 mg/unit, 253±87

mg/unit and 280±106 mg/unit respectively. These data supported her earlier hypothesis that the volume of supernatant plasma removed during the preparation affected the yield of fibrinogen.

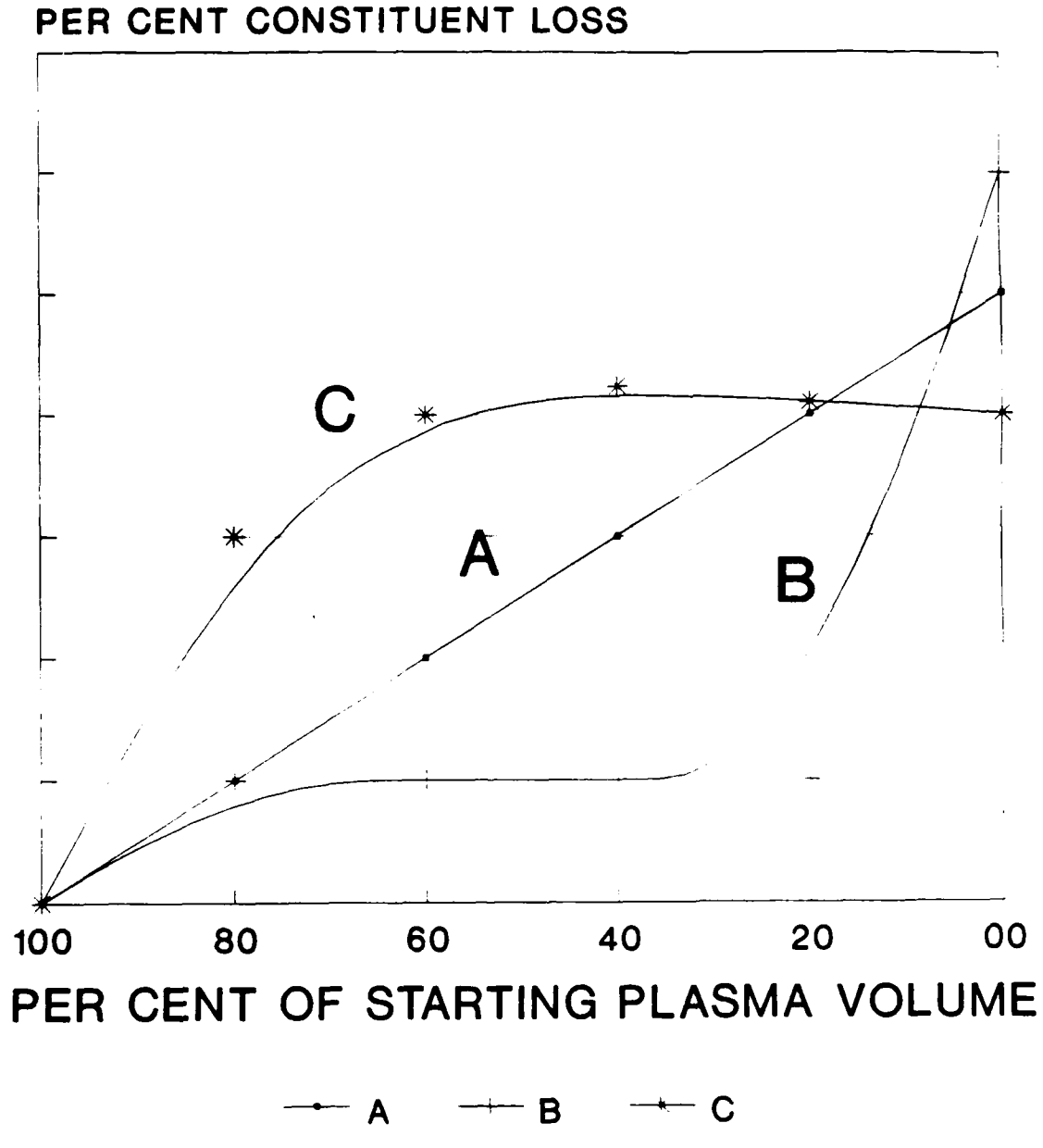
STATEMENT OF THE PROBLEM

Cryoprecipitate is the current preferred source of fibrinogen for the treatment of fibrinogen disorders.[46,50,51,61,62] Fibrinogen yields in cryoprecipitate vary, with recoveries reported from 28% to 71%.[76-80] The variables that influence the fibrinogen yield are not fully understood. The first factor that has been reported to influence fibrinogen yield is the final volume of cryoprecipitate. Hoffman has suggested a link between the residual supernatant volume and fibrinogen recovery[79,80] but an insufficient number of units have been tested to statistically prove a correlation between final volume and fibrinogen yield. Additionally, no studies have specifically investigated the kinetics of the loss of fibrinogen into the supernatant plasma as it is removed during cryoprecipitate preparation.

At least four possible mechanisms of fibrinogen loss could exist. Figure 4 pictorially illustrates three potential theoretical mechanisms. Fibrinogen could be lost into the supernatant plasma at a constant rate from the beginning to the end of its removal, ie., a simple linear relationship between accumulated supernatant volume and fibrinogen loss. A second possibility is that the fibrinogen loss could increase as the final volume is approached. Alternatively, the fibrinogen loss could be

FIGURE 4: Figure 4 represents three theoretical mechanisms of constituent loss in relationship to per cent of the total volume of the starting plasma unit. The higher per cent of starting plasma corresponds to the top of the units, the plasma which would flow off first, while the lower per cent volumes would flow off last. Curve A represents a simple linear relationship between constituent loss and per cent plasma volume. Curve B depicts a constant loss followed by a sharp increase as the final volume is approached. Curve C represents the opposite, a large early loss followed by a steady state.

THEORETICAL KINETICS OF CONSTITUENT LOSS



large early in the supernatant plasma removal, followed by a steady state or decline in fibrinogen loss throughout the remainder of the supernatant plasma removal. A fourth possibility that also must be considered is that the fibrinogen loss occurs sporadically throughout the supernatant plasma removal, with no specific pattern.

If the kinetics of fibrinogen loss in the cryoprecipitate preparation were known, procedure modifications might be made to improve fibrinogen yield in the cryoprecipitate. If the fibrinogen loss occurs at a constant rate or sporadically, then altering the final volume would be expected to be of little or no benefit. The same can be said if an early loss relationship exists. If, however the fibrinogen loss increases as the final volume is approached, ie., if most of the fibrinogen loss occurs late as depicted in Curve B of Figure 4, then increasing the final volume could possibly increase yield as suggested by Hoffman.[82,83]

The hypothesis to be tested in this study is that the fibrinogen loss occurs in a linear fashion, increasing as the supernatant plasma is removed. The null hypothesis is that no relationship, or a nonlinear one, exists between the supernatant plasma volume and fibrinogen loss.

To test the hypothesis, a study was designed that would measure the fibrinogen content in aliquots of supernatant plasma collected at set points in its removal. The

fibrinogen content of these aliquots would be compared to that of the original plasma unit and final cryoprecipitate. A regression analysis of the fibrinogen loss at the various points would then be performed to determine if the fibrinogen loss is linear across the supernatant volume removed.

Two methods of cryoprecipitate preparations are described in the Technical Manual of the American Association of Blood Banks (AABB). The fibrinogen loss in both methods were evaluated and the two methods compared. It was be imperative to evaluate factor VIII loss simultaneously for two reasons. First, the only standards that exist concerning the quality of cryoprecipitate preparation involve the factor VIII content. Second, should fibrinogen and factor VIII losses not parallel each other, attempts to maximize fibrinogen yields could adversely affect factor VIII yields. This would not be desired as cryoprecipitate is still used for the treatment of hemophilia A in some cases.

MATERIALS AND METHODS

Plasma Collection

A total of 123 units of whole blood (450 ± 45 ml) were collected from volunteer donors into blood collection bags containing 63 ml of citrate-phosphate-dextrose-adenine anticoagulant (CPDA-1) (CLX plastic bags, Cutter Biological, Berkeley, CA or PL 146 plastic bags, Fenwal Laboratories, Deerfield, IL) by the personnel of Blood Donor Centers at Ft Knox, KY, Ft Hood, TX, Keesler AFB, MS and Walter Reed Army Medical Center, Washington DC, using standard technique.[24,25] The whole blood units were centrifuged within six hours of collection and the plasma transferred to an integrally attached satellite bag and frozen at -18°C. Fresh frozen plasma (FFP) units were packed in dry ice for shipment to the testing laboratory where they were stored at -70°C until the time of cryoprecipitate preparation. Units of FFP were selected without regard for ABO group or Rh type and varied in age, but did not exceed twelve months.

Cryoprecipitate Preparation

Cryoprecipitate was prepared by two methods as described in the Technical Manual of AABB[25] and the fibrinogen and factor VIII losses in both methods were

studied. In one method, the quick-thaw method described in detail in Appendix A, the FFP was thawed in a 4°C circulating water bath until a slushy consistency was obtained. Thawing time was 45-60 minutes. The unit was then placed in a plasma expressor and the supernatant plasma removed, using the ice crystals as a filter to collect the precipitate. In the second method, the slow-thaw method described in detail in Appendix B, the FFP was thawed to completion at 4°C and centrifuged to pack the white precipitate to the bottom of the unit. Thawing time was 18-20 hours. The unit was suspended, inverted, and the supernatant plasma allowed to flow from the unit by gravity.

Aliquot Collection

Aliquots were collected at predetermined points during the supernatant plasma removal. Since the total volume of FFP units vary, aliquots could not be collected after set volumes of supernatant plasma was removed, as this would represent different portions of the plasma for each unit and the linear regression model could not be used. To circumvent this problem, the aliquot collection points were normalized to the total volume of the units, by collecting aliquots at set percentages of the total starting plasma volume of each unit. The percentages selected for aliquot collection were 80%, 60%, 40% and 20% to cover the range of

supernatant volume removed. One can think of these points as relating to the supernatant plasma height in the original unit, the 80% point being near the top and the 20% point being near the bottom of the unit. Consequently, the plasma at the 80% point flows off first and the 20% last.

A sample of original plasma was obtained from an integrally attached segment removed from each unit of FFP prior to thawing to serve as the 100% point for recovery studies. All aliquots were stored at -70°C after collection until time of testing.

Assays

Cryoprecipitate units were thawed in a 37°C circulating water bath for 15 minutes with a plastic overwrap. Aliquots were thawed in the same manner, however, no overwrap was used. In all instances, factor VIII assays were performed first to minimize in-vitro degradation of the factor VIII activity.

Fibrinogen content was determined by the Thrombin Time test, which measures the amount of fibrinogen converted to fibrin by the enzyme thrombin.[81] A 1:10 dilution of plasma was made with Owren's Veronal buffer, pH 7.4±0.1 (formula detailed in Appendix C). This dilution was mixed with thrombin reagent of approximately 100 NIH units (Organon Teknika, Durham NC) and the time required for a clot to form

was measured. This time was compared to a standard curve prepared using a fibrinogen reference plasma of known concentration (Organon Teknika, Durham NC). Fibrinogen concentration was measured in mg/100 ml (mg/dl).

Factor VIII assays were performed using the one-stage activated partial thromboplastin time test (APTT) test.[82] Test plasma was mixed in equal volumes with immunoadsorbed factor VIII deficient plasma (Organon Teknika, Durham NC). A 1:10 dilution of the test plasma-factor VIII deficient plasma mixture was made with imidazole buffered saline, pH 7.3±0.1 (formula detailed in Appendix C). This dilution was activated with platelet factor 3 reagent (Automated APTT reagent, Organon Teknika, Durham NC) for three minutes, followed by the addition of 0.25 M calcium chloride (Organon Teknika, Durham NC). The time required for clot formation was measured and compared against a standard curve prepared with known dilutions of normal control plasma (Organon Teknika, Durham NC). A 1:10 dilution of normal control plasma was assigned an activity of 100%. Factor VIII activity was measured in per cent factor VIII/ml. All testing was performed on a semi-automated, photo-optic coagulation analyzer (Coag-A-Mate XC, General Diagnostics, Durham NC).

Calculation of Per Cent Recoveries

Fibrinogen concentration, measured in mg/dl, was converted to mg/ml by dividing by 100. The total amount of fibrinogen in the FFP and cryoprecipitate was calculated by multiplying the concentrations of fibrinogen, in mg/ml, by the total volumes of the FFP and cryoprecipitate, in ml. Per cent recoveries were determined by dividing the total amount of fibrinogen in the cryoprecipitate by the amount in the FFP and multiplying by 100.

The total number of units of factor VIII activity in both the FFP and cryoprecipitate were calculated by multiplying the plasma volumes, in ml, by the factor VIII activity measured in units of factor VIII/ml (U/ml). Per cent recoveries were determined by dividing the units of factor VIII in the cryoprecipitate by that in the FFP and multiplying by 100.

Statistical Analysis

This study was designed to determine the relationship between two variables, volume of supernatant plasma removed and fibrinogen loss into the supernatant. The statistical model chosen to test whether a relationship exists between the two variables is the linear regression model.[83,84,85]

The regression line of Y on X and the associated p-value

was determined by the method of least squares.[85] The Wilk-Shapiro test was used to determine if the Y values were randomly distributed. If the ıta were not normally distributed, then logarithmic transformations were performed to establish a true normal distribution.

All means presented in this report are arithmetic means and reported in the format mean ± one standard deviation. One-way analysis of variance was used to compare mean values of different subpopulations and calculate probability values (p-values) to determine the significance of the difference between the two. The level of significance used in this study was 0.05, $p<0.05$ considered significant for rejection of the null hypothesis.

Statistical analysis was accomplished using a commercially available computer statistical package (Statistix II, NH Analytical Software, Roseville, MN).

RESULTS

Summary Statistics

A total of 123 units of cryoprecipitate were prepared, 62 by the quick thaw method and 61 by the slow thaw method. The total volume of the FFP units used to prepare the cryoprecipitate was 217.4±26.1 ml (mean±standard deviation) (range, 140-314 ml). The total amount of fibrinogen in those units was 457.3±115.9 mg/unit (range, 234.7-763.2) and factor VIII was 157.1±70.9 Units of factor VIII activity/unit of cryoprecipitate (range 32.0-443.9). Table 2 lists the summary data for the FFP units by method. Descriptive data of the cryoprecipitate units prepared by each method are given in Table 3. In both tables the p-values provide an assessment for the comparison of the quick thaw method with the slow thaw method. P-values were obtained by one-way analysis of variance with two treatments.

Fibrinogen Loss Into Supernatant Plasma

The mean fibrinogen loss into the supernatant plasma at each aliquot is given by method in Table 4. The values are listed as per cents of the total fibrinogen in the original starting plasma. The p-values were calculated by a one-way

TABLE 2: STARTING PLASMA STATISTICAL SUMMARY BY METHOD*

	QUICK THAW	SLOW THAW	p#
Total Volume (ml)	219.9 ± 29.5	215.1 ± 22.1	0.37
Total Fibrinogen (mg)	455.8 ± 113.5	460.1 ± 120.1	0.39
Total Factor VIII (U)	150.7 ± 58.6	163.1 ± 81.8	0.39
n	62	61	-

* Results reported as mean ± one standard deviation.
\# p-values calculated by one-way analysis of variance and predict variation of volume or content between the two methods.

TABLE 3: CRYOPRECIPITATE STATISTICAL SUMMARY BY METHOD*

	QUICK THAW	SLOW THAW	p#
Final Volume (ml)	27.5 ± 7.5	14.1 ± 4.0	<0.01
Fibrinogen (mg)	91.1 ± 35.2	185.2 ± 59.9	<0.01
Fibrinogen Yield (%)	20.8 ± 9.5	41.5 ± 11.8	<0.01
Factor VIII (U)	50.1 ± 30.5	82.1 ± 40.4	<0.01
Factor VIII Yield (%)	34.6 ± 17.7	54.1 ± 21.5	<0.01
n	62	61	-

* Results reported as mean ± one standard deviation.
\# p-values calculated by one-way analysis of variance and predict variation of volume or content between the two methods.

TABLE 4: PER CENT FIBRINOGEN LOSS IN SUPERNATANT PLASMA BY METHOD*#

ALIQUOT	QUICK THAW	SLOW THAW
80%	1.01 ± 0.24	0.59 ± 0.17
60%	0.97 ± 0.24	0.60 ± 0.19
40%	0.93 ± 0.24	0.62 ± 0.26
20%	0.91 ± 0.24	0.60 ± 0.19
p@	0.07	0.88
n	248	232

* Results reported as mean ± one standard deviation.
\# Per cent of total fibrinogen in starting plasma.
@ p-values calculated by one-way analysis of variance and predict variation in content between the aliquots.

analysis of variance test with four treatments. The number of tests listed corresponds to the total number of aliquots tested.

Regression analysis of the log transformation of per cent fibrinogen loss across the aliquots was performed. Because the raw data were not normally distributed as determined by Wilk-Shapiro analysis, log transformation of percentiles was performed, normalizing the data, satisfying the distributional assumptions of the linear regression model. Multiple linear regression analysis was also performed to compare the slopes of the regression lines of fibrinogen loss by the two methods. The slope of the linear regression of log per cent fibrinogen loss against aliquot collection points, representing the volume of supernatant plasma, for the quick thaw method was $-8.15X10^{-2}$ ($p<0.01$), and $1.00x10^{-4}$ ($p>0.77$) for the slow thaw method. The difference between the two methods was significant, $p<0.04$ as determined by multiple regression analysis.

Factor VIII Loss in Supernatant Plasma

The factor VIII loss into the supernatant plasma at each aliquot is given in Table 5. Data presented in Table 5 are mean values of the per cent of total factor VIII in the original starting plasma lost at each aliquot. The p-values were calculated by a one-way analysis of variance and the

TABLE 5: PER CENT FACTOR VIII LOSS IN SUPERNATANT PLASMA BY METHOD*#

ALIQUOT	QUICK THAW	SLOW THAW
80%	0.77 ± 0.33	0.24 ± 0.10
60%	0.78 ± 0.33	0.24 ± 0.11
40%	0.71 ± 0.36	0.24 ± 0.13
20%	0.73 ± 0.41	0.24 ± 0.12
p@	0.66	0.99
n	248	232

* Results reported as mean ± one standard deviation.
\# Per cent of total factor VIII of starting plasma.
@ p-values calculated by one-way analysis of variance and predict variation in content between aliquots.

total number of aliquots tested is given as n.

As with the fibrinogen data, per cent factor VIII was log transformed. The linear regression of the log per cent of factor VIII loss against the supernatant plasma volume, represented by the aliquots, for the quick thaw method had a slope of -8.78×10^{-4} ($p>0.11$). The slope of the slow thaw regression line was -2.64×10^{-4} ($p>0.62$). The difference between the two was not significant ($p>0.43$), as determined by multiple regression analysis.

Agreement With Model

The effectiveness of the model at accounting for all of the fibrinogen and factor VIII in the original plasma was evaluated. The mean per cent losses of fibrinogen and factor VIII at each aliquot did not differ significantly from each other as determined by one-way analysis of variance. Therefore, the mean per cent loss for all four aliquots was used to calculate the predicted total amount of fibrinogen and factor VIII lost into the supernatant plasma. The total fibrinogen and factor VIII in the supernatant plasma was added to the totals recovered in the cryoprecipitate and the sum compared to the totals in the starting plasma. The per cents of the total fibrinogen and factor VIII accounted for in the supernatant plasma and cryoprecipitate are listed in Table 6.

TABLE 6: PREDICTED PER CENT FIBRINOGEN AND FACTOR VIII ACCOUNTED FOR BY THE MODEL*#

	QUICK THAW	SLOW THAW
Fibrinogen	111	67
Factor VIII	108	74

* Results reported as per cent of total fibrinogen and factor VIII of original starting plasma.
\# Mean values were used in calculations.

DISCUSSION

Starting Plasma Summary Statistics

As was seen in Table 2, the starting plasma units used to prepare cryoprecipitate by the two methods did not differ significantly in total volume, fibrinogen nor factor VIII content. Hence, it was expected that any differences in the results between the two methods were due to differences in the procedures, since the starting materials were equivalent. The cryoprecipitate units, on the other hand, differed significantly in all three parameters between the two methods.

Cryoprecipitate Summary Statistics

The difference in the mean final volumes, 27.5 ml for the quick thaw versus 14.1 ml for the slow thaw, was significant ($p<0.01$). The difference in the final volume may largely be a result of the unthawed ice crystals that remained in the units prepared by the quick thaw method, that when thawed to completion, would add the additional volume. On a practical note, it was much easier to control the final volumes of the units prepared by the slow thaw method, as is evidenced by the standard deviations of the final volumes, only 4.0 ml for the slow thaw compared to

7.5 ml for the quick thaw.

Before proceeding, a clarification of terminology that will be used in the remainder of this manuscript should be made. Recovery refers to that amount, or per cent, of either fibrinogen or factor VIII that was recovered in the final cryoprecipitate. This represents the transfusable product. Conversely, loss refers to the fibrinogen or factor VIII that is "lost" into the supernatant plasma and therefore not found in the cryoprecipitate.

Fibrinogen Loss Into Supernatant Plasma

There was a significant difference between the fibrinogen content and per cent recovery of fibrinogen in the cryoprecipitate between the two methods, $p<0.01$. The mean fibrinogen content of the cryoprecipitate prepared by the slow thaw method, 185.2 mg/unit, was twice that of the quick thaw method, only 91.1 mg/unit. The recoveries had a similar relationship when compared; 41.5% for the slow thaw method versus 20.8% for the quick thaw. These data are compared with previously published reports in Table 7. The variability in fibrinogen yield of cryoprecipitate is readily apparent when these data are examined. The fibrinogen yield in the slow thaw procedure used in this method is somewhat lower than most of the other reports, while the quick thaw was even lower. One possible contribu-

TABLE 7: COMPARISON OF FIBRINOGEN DATA WITH PREVIOUS PUBLISHED STUDIES

INVESTIGATOR	MEAN (MG/UNIT)*	NO OF UNITS
Soloway/Bereznak[77]	250 ± 30	--**
Oka[76]	285 - 300#	10
Prowse/McGill[78]		
Mason Thaw Siphon	225 ± 12	45
Quick-Thaw##	155 ± 12	25
Slow-Thaw	139 ± 24	25
Ness[75]	266 (63-417)@	88
Hoffman[79]		
Low Volume	101 ± 48	23
Reg Volume	142 ± 50	8
Hoffman[80]		
Quick Thaw (Low Vol)##,@@	191 ± 37	11
Quick Thaw (Reg Vol)##	243 ± 87	16
Slow Thaw (Reg Vol)	280 ± 106	20
Current Study		
Quick Thaw	91 ± 35	62
Slow Thaw	185 ± 59	61

* Data presented as mean ± standard deviation
** Determined by in-vivo studies following cryoprecipitate infusion.
\# Range of data given, no mean.
Quick thaw procedure differed from this study in that FFP units were centrifuged after thawing prior to removal of supernatant plasma.
@ No standard deviation given.
@@ Low volume units, mean volume of 4 ml.

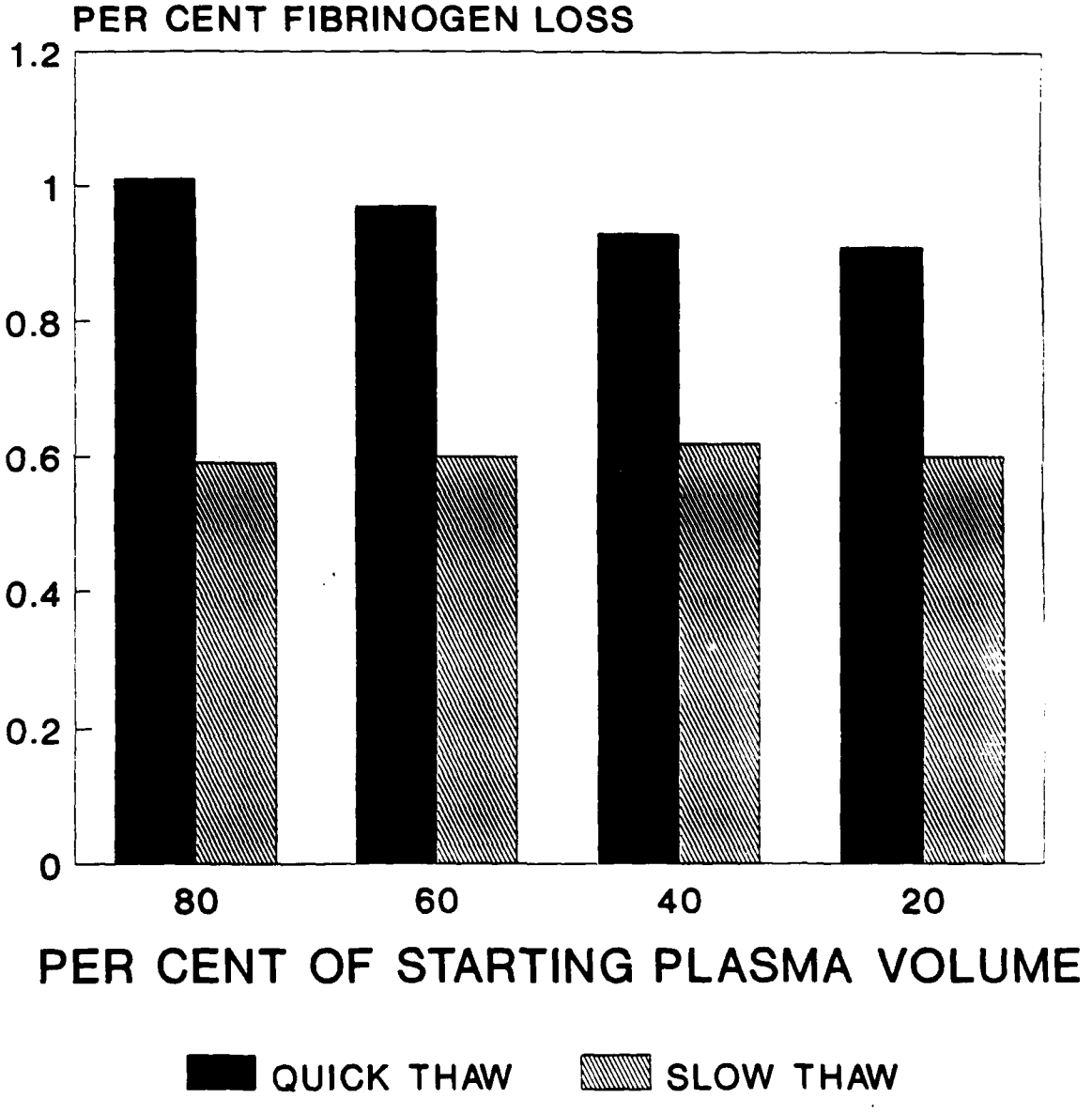
ting factor to the lower values in the slow thaw procedure was the slight modification to the procedure that was made to accomplish this study; i.e. the collection of aliquots of supernatant plasma as it was removed. Normally, the supernatant plasma flows off rapidly and uninterrupted, while in this study that flow was stopped momentarily to collect the aliquots, increasing the time required for supernatant removal. This allowed the supernatant plasma to remain in contact with the cryoprecipitate longer, possibly increasing the loss.

Figure 5 plots the mean per cent loss of fibrinogen at each aliquot for the quick thaw and slow thaw methods. There appeared to be a rather constant loss across the aliquots for the slow thaw method, which suggests a declining fibrinogen loss as collection of supernatant progresses, although only slightly (Figure 5). One-way analysis of variance of the data showed no significant difference between the mean values at the different aliquots ($p>.05$) within both methods despite the apparent linear relationship in the quick thaw method. The loss for the quick thaw method was slightly higher than the slow thaw method, as was verified by the lower recoveries in the cryoprecipitate prepared by that method.

Linear regression of the log of the per cent loss of fibrinogen against per cent volume of supernatant plasma was performed. The resulting slopes for the quick thaw and slow

FIGURE 5: The fibrinogen loss into the supernatant plasma is plotted against the per cent of starting plasma volume. The 80% point corresponds to the top of the unit of cryoprecipitate and represents the supernatant plasma that would flow from the unit first. Conversely, the 20% point is located in the bottom portion of the unit and flows off last.

FIBRINOGEN LOSS INTO SUPERNATANT PLASMA BY METHOD



thaw methods were $-8.15x10^{-2}$ and $1.00x20^{-4}$ respectively. The slope of the fibrinogen loss regression line by the quick thaw method was significant ($p<0.01$). The slope for the slow thaw was not. From the data, it would appear that there exists a linear relationship between the loss of fibrinogen and supernatant plasma volume in the quick thaw method. The negative slope suggests an inverse linear relationship. Hence, the higher the per cent of total starting plasma volume, the higher the fibrinogen loss. Or stated another way, the higher in the unit, the earlier the runoff, the higher the fibrinogen loss. No such relationship exists in the slow thaw method. The null hypothesis that $\beta=0$ was rejected for the quick thaw and was not rejected for the slow thaw. Multiple regression of both methods demonstrated that the difference in the slopes between the methods was significant ($p<0.04$). Since the difference is so small it would not be expected to be of any practical significance.

Factor VIII Loss Into The Supernatant Plasma

As with fibrinogen, a significant difference was detected between the recovery of factor VIII in the cryoprecipitate in the two methods. The slow thaw method met the minimum standard set by the FDA of 80 Units of factor VIII/unit of cryoprecipitate, with a mean of 82.1

Units of factor VIII/ unit of cryoprecipitate.[25] The cryoprecipitate prepared by the quick thaw method, on the other hand, did not meet the standard with a mean factor VIII content of only 50.1 Units per unit of cryoprecipitate. The AABB requirement is that 75% of the cryoprecipitate units prepared must contain at least 80 Units of factor VIII.[23] The cryoprecipitate prepared by either method did not meet this standard, only 19% of the quick thaw units met the standard and 44% of the slow thaw contained the minimum. The corresponding mean recoveries of factor VIII were 54.1% for the slow thaw method and 34.6% for the quick thaw. The factor VIII recoveries compared with those of previously published reports; reported recoveries ranging from 38% to 64%.[31,32,34,35,37,50,51]

Modifications of the quick thaw method[78,79,80] have been reported in the literature to increase the yield of fibrinogen and factor VIII. The most frequently used modification being the thawed product is centrifuged prior to removal of supernatant plasma and the supernatant removed by inversion, as in the slow thaw method. The centrifugation step, which packs the cryoprecipitate, is likely the most crucial part, as the cryoprecipitate could be visually observed escaping into the supernatant plasma as the supernatant was removed in the quick thaw method used in this study. The ice crystals were not fully effective at retaining the smaller bits of cryoprecipitate in the method

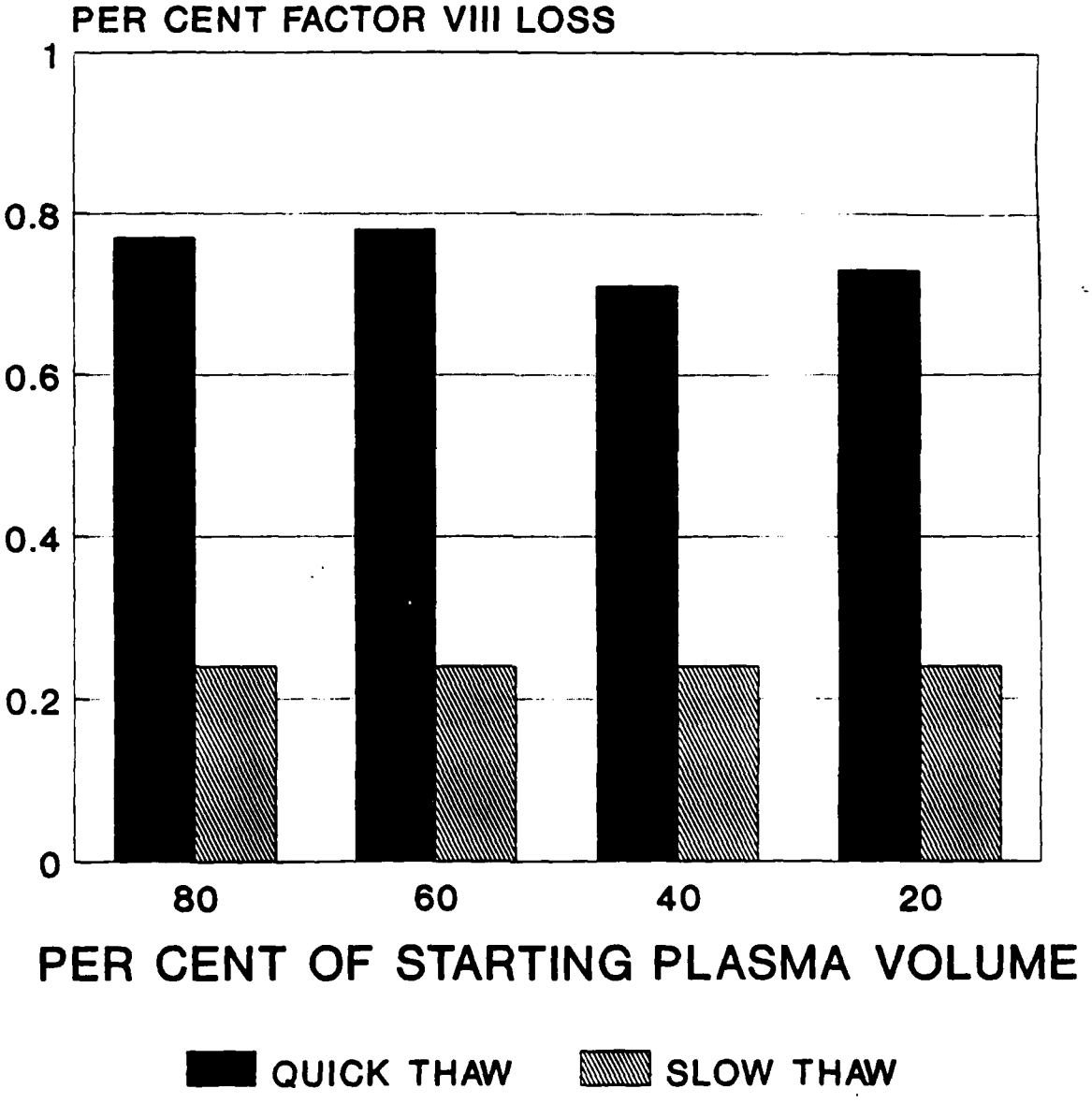
as it is described in this study. This is an area which requires further study. Additionally, as noted previously, the procedure modification required to collect the aliquots increased the amount of time required to prepare the cryoprecipitate by the slow thaw method. This may also have affected the factor VIII recoveries in the cryoprecipitate. The increased time required should not have been sufficient for factor VIII degradation to occur, but allowed increased contact between the supernatant with the precipitate.

The mean per cent loss of factor VIII is plotted against the aliquot collection points in Figure 6 for the two methods. As with fibrinogen loss in the slow thaw method, the loss of factor VIII appears to be constant across the aliquots in both methods. This is supported by one-way analysis of variance which showed no significant difference between the means within the methods. The loss in the quick thaw method was slightly higher as was expected by the lower cryoprecipitate recoveries.

Linear regression analysis of log per cent factor VIII loss against supernatant plasma volume yielded a slope of -8.78×10^{-4} for the quick thaw method and -2.64×10^{-4} for the slow thaw. Neither slope was significant ($p>0.05$). The difference between the slopes for the two methods as analyzed by multiple linear regression, likewise, was not significant. Therefore, we failed to reject the null hypothesis that $\beta=0$ in both methods indicating a statisti-

FIGURE 6: The factor VIII loss into the supernatant plasma is plotted against the per cent of total starting plasma volume. The 80% aliquot is near the top of the unit and would flow from the unit first. The 20% aliquot is near the bottom and would flow off last.

FACTOR VIII LOSS INTO SUPERNATANT PLASMA BY METHOD



cally significant linear relationship does not exist between factor VIII loss and supernatant plasma volume.

Agreement With Model

The effectiveness of this study design in accounting for the total amount of fibrinogen and factor VIII in the starting plasma was evaluated. The model was able to account for all of the fibrinogen and factor VIII in the starting plasma used in the quick thaw method, predicted recoveries of 111% and 105% respectively. This is within an anticipated error of 15% due to measurement error and the assumption that the mean fibrinogen and factor VIII of the aliquots represented the true concentration.

In the slow thaw method, only 67% of the fibrinogen and 74% of the factor VIII could be accounted for by the model. This suggests that the losses of fibrinogen and factor VIII are higher at some other point in the supernatant plasma. It is possible, but highly unlikely, that the fibrinogen or factor VIII losses were higher in between the aliquots collected, i.e. a spike or surge of fibrinogen or factor VIII went undetected. What is more likely, is that the fibrinogen and factor VIII losses increased between the 20% collection point and the final cryoprecipitate volume, which represents 7% to 12% of the starting plasma volume. This would support Hoffman's theory that the fibrinogen loss

increases as the final volume is approached. This in fact, would support the earlier suggestion that the lower recoveries in this study were in part due to the increased cryoprecipitate preparation time required for aliquot collection. If the loss is much higher in the interval between the 20% point and final cryoprecipitate volume, then increasing the time the supernatant remains on the precipitate could lead to diminished yields, because it is this last ten per cent of residual supernatant plasma which has the longest and closest contact to the precipitate.

If indeed the constituent loss increases between the 20% point and final cryoprecipitate volume a potential follow-up study would be to collect aliquots at very frequent intervals between these points and measure the fibrinogen and factor VIII content of each aliquot. Linear regression of the data would again be an appropriate statistical analysis tool. Two likely outcomes are anticipated, either a linear relationship with a significant slope or an exponential rise in fibrinogen loss as the final volume is approached would be observed.

Summary

This study revealed that of the two methods of cryoprecipitate preparation detailed in the Technical Manual of the AABB, the quick thaw method and slow thaw method, the

slow thaw is far superior in both recovery of fibrinogen and factor VIII. Additionally, the slow thaw method results in a product of significantly lower final volume which is of clinical importance, as volume overload is a serious side effect of repeated transfusion of plasma products.[50] Furthermore, the final volume was much more consistent between units.

The question that this study sought to answer was whether there was a linear relationship between the volume of supernatant plasma removal and loss of fibrinogen. For the quick thaw procedure a linear relationship exists between fibrinogen loss and supernatant volume. The relationship being a slightly higher initial per cent loss, with a linear decline, though the rate of that decline was quite small and of little or no practical value. Some relationship other than linear existed for factor VIII loss.

For the slow thaw method, the question was only half answered. There was no linear relationship demonstrated to exists between the volume of supernatant plasma removed and the loss of fibrinogen or factor VIII; furthermore, the model failed to account for 33% and 26% of the fibrinogen and factor VIII, respectively. The relationship between the volume of supernatant plasma and fibrinogen loss requires closer evaluation of losses which occur between the 20% point and final cryoprecipitate. Future studies should be undertaken to evaluate what that relationship is and what

practical use can be made of the information to improve the yield of fibrinogen and factor VIII.

REFERENCES

1. Corriveau DM. Major elements of hemostasis. In: Corriveau DM, Fritsma GA, eds. Hemostasis and thrombosis in the clinical laboratory. Philadelphia: JB Lippincott, 1988:1-33.

2. Colman RW, Marder VJ, Salzman EW, Hirsh J. Overview of hemostasis. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis, basic principles and clinical practice. 2nd ed. Philadelphia: JB Lippincott, 1987:3-17.

3. Stemerman MB. Anatomy of the blood vessel wall. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis, basic principles and clinical practice. 2nd ed. Philadelphia: JB Lippincott, 1987:773-780.

4. Corriveau DM. Plasma proteins: factors of the hemostatic mechanism. In: Corriveau DM, Fritsma GA, eds. hemostasis and thrombosis in the clinical laboratory. Philadelphia: JB Lippincott, 1988;34-66.

5. Murano G. A basic outline of blood coagulation. Semin Thromb Hemost 1980;7:140-162.

6. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. Science 1964;145:1310-1311.

7. MacFarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. Nature 1964;202:498-499.

8. Osterud B. Activation pathways of the coagulation system in normal hemostasis. Scand J Haematol 1984;32:337-345.

9. Hemker HC, Kahn MJP. Reaction sequence of blood coagulation. Nature 1967;215:1201-1202.

10. Colman RW. Factor V. Prog Hemost Thromb 1974;3:109-143.

11. Kane WH, Davie EW. Blood coagulation factors V and VIII: structural and functional similarities and their relationship to hemorrhagic and thrombotic disorders. Blood 1988;71:539-555.

12. Wilner GD, Nossel HL, LeRoy EC. Activation of hageman factor by collagen. J Clin Invest 1968;49:2608-2615.

13. Kaplan AP. Initiation of the intrinsic coagulation and fibrinolytic pathways of man: the role of surfaces, hageman factor, prekallikrein, high-molecular-weight kininogen and factor XI. Prog Hemost Thromb 1978;4:127-175.

14. Kaplan AP, Silverberg M. The coagulation-kinin pathway of human plasma. Blood 1987;70:1-15.

15. Mandle RJ, Colman RW, Kaplan AP. Identification of prekallikrein and high-molecular-weight kininogen as a complex in human plasma. Proc Natl Acad Sci USA 1976;73:4179-4183.

16. Ratnoff OD, Davie EW. The activation of christmas factor (factor IX) by activated plasma thromboplastin antecedent (activated factor XI). Biochem 1962;1:677-685.

17. Zur M, Nemerson Y. Kinetics of factor IX activation via the extrinsic pathway: dependence of K_m on tissue factor. J Biol Chem 1980;255:5703-5707.

18. Osterud B, Rapaport SI. Function of formed activator by antibodies to factor VIII and to factor IX. Biochem 1970;9:1854-1861.

19. Hoyer LW. Molecular pathology and immunology of factor VIII (hemophilia A and factor VIII inhibitors). Human Pathology 1987;18:153-161.

20. Herman J. McDonagh J. Fibrin: structure and interactions. Semin Thromb Hemost 1982;8:11-24.

21. Lusher JM. Replacement therapy for congenital and acquired disorders of blood coagulation - available products. In: Garraty G, ed. Current concepts in transfusion therapy. Arlington, VA: American Association of Blood Banks, 1985:27-49.

22. Snyder EL, ed. Blood transfusion therapy: a physician's handbook. 2nd ed. Arlington, VA: American Association of Blood Banks, 1987:23.

23. Holland PV, Schmidt PJ, eds. Standards for blood banks and transfusion services. 12th ed. Arlington, VA: American Association of Blood Banks, 1987:12-20.

24. Widmann FK, ed. Technical Manual. 9th ed. Arlington, VA: American Association of Blood Banks, 1985:43-57.

25. US Department of Health and Human Services, Food and Drug Administration. The code of federal regulations, 21 CFR 640.54. Apr 88 ed. Washington, DC: US Government Printing Office, 1988:128-129.

26. Pool JG, Shannon AE. Production of high-potency concentrates of antihemophilic globulin in a closed-bag system. N Engl J Med 1965;273:1443-1447.

27. Bloom AL. Introduction. In Bloom AL, ed. The hemophilias. Edinburgh: Chruchill Livingstone, 1982:1-19.

28. Aronson DL. Factor VIII (antihemophilic globulin). Semin Thromb Hemost 1979;VI:12-27.

29. Johnson AJ, Mathews RW, Fulton AJ. Fractionation of factor VIII and IX-an overview. Scand J Haematol [Supp] 1984;40:513-524.

30. Pool JG. Cryoprecipitate quality and supply. Transfusion 1975;15:305-306.

31. Burka ER, Puffer T, Martinez J. The influence of donor characteristics and preparation methods on the potency of human cryoprecipitate. Transfusion 1975;15:323-328.

32. Kasper CK, Muhre BA, McDonald JD, Nakasko Y, Feinstein. Determinants of factor VIII recovery in cryoprecipitate. Transfusion 1975;15:312-322.

33. Vermeer C, Soute BAM, Ates G, Brummelhuis HGJ. Contributions to the optimal use of human blood. VI. Increase of the yield of factor VIII in four-donor cryoprecipitate by an improved processing of blood and plasma. Vox Sang 1976;30:1-22.

34. Slichter SJ, Counts RB, Henderson R, Harker LA. Preparation of cryoprecipitated factor VIII concentrates. Transfusion 1976;16:616-626.

35. Rock G, Tittley P. Variations in cryoprecipitate production. Transfusion 1977;17:50-53.

36. Working Party of the Regional Transfusion Directors Committee. Variables involved in cryoprecipitate production and their effect on factor VIII activity. Br J Haematol 1978;43:287-295.

37. Rock GA, Tittley P. The effect of temperature variations on cryoprecipitate. Transfusion 1979;19:86-89.

38. Farrugia A, Prowse C. Studies on the procurement of

blood coagulation factor VIII: effects of plasma freezing rate and storage conditions on cryoprecipitate quality. J Clin Pathol 1985;38:433-437.

39. Marder VJ, Mannucci PM, Firkin BG, Hoyer LW, Meyer D. Standard nomenclature for factor VIII and von willebrand factor: a recommendation by the international committee on thrombosis and haemostasis. Thromb Hemost 1985;54:871-872.

40. Vehar GA, Davie EW. Preparation and properties of bovine factor VIII (antihemophilic factor). Biochem 1980;19:401-410.

41. Fass DN, Knutson GJ, Katzmann JA. Monoclonal antibodies to porcine factor VIII coagulant and their use in the isolation of active coagulant protein. Blood 1982;59:594-600.

42. Knutson GJ, Fass DN. Porcine factor VIII:C prepared by affinity interaction with von willebrand factor and heterologous antibodies: sodium dodecyl sulfate polyacrylamide gel analysis. Blood 1982;59:615-624.

43. Fulcher CA, ZImmerman TS. Characterization of the human factor VIII procoagulant protein with a heterologous precipitating antibody. Proc Natl Acad Sci USA,1982;79:1648-1652.

44. Vehar GA, Keyt B, Eaton D. Structure of human factor VIII. Nature 1984;312:337-342.

45. Toole JJ, Knopf JL, Wozney JM, et al. Molecular cloning of a cDNA encoding human antihemophilic factor. Nature 1984;312:342-347.

46. Wood WI, Capon DJ, Simonsen CC, et al. Expression of active human factor VIII from recombinant DNA clones. Nature 1984;312:342-347.

47. Gitscher J, Wood WI, Goralka TM, et al. Characterization of human factor VIII gene. Nature 1984;312:326-330.

48. Fulcher CA, Roberts JR, Zimmerman TS. Thrombin proteolysis of purified factor VIII procoagulant protein: correlation of activation with generation of a specific polypeptide. Blood 1983;61:807-811.

49. Levine PH. Clinical manifestations and therapy of hemophilia A and B. In. Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 2nd ed. Philadelphia: JB Lippincott, 1987:97-111.

50. Thompson AR, Harker LA. Manual hemostasis and thrombosis. 3rd ed. Philadelphia: FA Davis, 1983:99-113.

51. Hall CE, Slayter HS. The fibrinogen molecule: its size, shape, and mode of polymerization. J Biophy Cytol 1959;5:11-15.

52. Tooney NM, Cohen C. Microcrystals of a modified fibrinogen. Nature 1972;237:23-25.

53. Cohen C, Tooney NM. Crystallization of a modified fibrinogen. Nature 1974;251:659-660.

54. Doolittle RF, Goldbaum DM, Doolittle LR. Designation of sequences involved in the "coiled-coil" interdomainal connection in fibrinogen: construction of an atomic scale model. J Mol Biol 1978;120:311-325.

55. Weisel JW, Stauffacher CV, Bullitt E, Cohen C. A model for fibrinogen: domains and sequences. Science 1985;230:1387-1391.

56. Blomback B, Blomback M. The molecular structure of fibrinogen. Ann NY Acad Sci 1972;202:77-97.

57. Henschen A, Lottspeich F, Kehl M, Southan C. Covalent structure of fibrinogen. Ann NY Acad Sci 1983;408:28-43.

58. Hantgan RR, Francis CW, Scheraga HA, Marder VJ. Fibrinogen structure and physiology. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 2nd ed, Philadelphia: JB Lippincott, 1987;269-288.

59. Ratnoff OD. Disordered hemostasis in hepatic disease. In: Schiff L, Schiff ER, eds. Diseases of the liver. 6th ed, Philadelphia: JB Lippincott, 1987;187-207.

60. Collen D, Tytgat GN, Claeys H, Piessens R. Metabolism and distribution of fibrinogen: fibrinogen turnover in physiological conditions in humans. Br J Haematol 1972;22:681-700.

61. Reeve EB, Franks JJ. Fibrinogen synthesis, distribution and degradation. Semim Throm Hemost 1974;1:129-172.

62. McDonagh H, Carrel N. Disorders of fibrinogen structure and function. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 2nd ed. Philadelphia: JB Lippincott, 1987;301-312.

63. Bove JR. Fibrinogen - is the benefit worth the risk? Transfusion 1978;18:129-136.

64. Menache D. Congenital fibrinogen abnormalities. Ann NY Acad Sci 1983;408:121-125.

65. Marder VJ, Martin SE, Francis CW, Colman RF. Consumptive thrombohemorrhagic disorders. In: Colman RW, Hirsh J, Marder VJ, Salzman EW, eds. Hemostasis and thrombosis: basic principles and clinical practice. 2nd ed. Philidephia: JB Lippincott, 1987:975-1015.

66. Ockelford PA, Carter CS. Disseminated intravascular coagulation: the application and utility of diagnostic tests. Semin Thromb Hemost 1982;8:198-216.

67. Cohn EJ, Strong LE, Hughes WL, Mulford DJ, Ashworth JN, Melin AM, Taylor HL. Preparation and properties of serum and plasma proteins. IV. a system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. J Am Chem Soc 1946;68:459-475.

68. Aronson DL, Finlayson JS. Historical and future therapeutic plasma derivatives: epilogue. Semin Thromb Hemost 1980;VI:121-139.

69. McMillan CW, Diamond LK, Surgenor DM. Treatment of classic hemophilia: the use of fibrinogen rich in factor VIII for hemorrhage and for surgery. N Engl J Med 1961;265:224-230.

70. Mainwarning RL, Brueckner GG. Fibrinogen transmitted hepatitis: a controlled study. JAMA 1966;195:437-441.

71. Cronberg S, Belfrage S, Nilsson IM. Fibrinogen-transmitted hepatitis. Lancet 1963;I:967-969.

72. Gerety RJ, Aronson DL. Plasma derivatives and viral hepatitis. Transfusion 1982;22:347-351.

73. Thomas KB, McGrath KM, Taylor M, Young IF, Herrington RW, Schiff P. Effect of virucidal heat treatment on proteins in human factor VIII concentrates. Transfusion 1988;28:8-13.

74. Hile JP. Fibrinogen (human). revocation of licenses. Fed Register 1978;43:1131-1132.

75. Ness PM, Perkins HA. Cryoprecipitate as a reliable source of fibrinogen replacement. JAMA 1979;241:1690-1691.

76. Oka WT, Gluick HI, Mauer AM, Hoxworth PI. Antihemophilic factor - nineteen months' experience in its production and use. Am J Med Technol 1968;34:373-387.

77. Soloway HB, Bereznak CE. Plasma fibrinogen levels following cryoprecipitate infusion. Transfusion 1970;10:326-328.

78. Prowse CV, McGill A. Evaluation of the 'Mason' (continuous-thaw-siphon) method for cryoprecipitate production. Vox Sang 1979;37:235-243.

79. Hoffman M, Koepke JA, Widman FK. Fibrinogen content of low-volume cryoprecipitate. Transfusion 1987;27:356-358.

80. Hoffman M. Effect of preparation method on the fibrinogen and von Willebrand's factor content of cryoprecipitate [Abstract]. Transfusion 1988;28:(Suppl):17S

81. Ratnoff OD, Menzie AB. A new method for the determination of fibrinogen in small samples of plasma. J Lab Clin Med 1951;37:316-320.

82. Lenahan JG, Smith K. Hemostasis. 18th ed. Durham NC: Organon Teknika, 1986:26-29.

83. Remington RD, Shork MA. Statistics with applications to the biological and health sciences. Englewood Cliffs, MJ: Prentice-Hall, 1985:235-258.

84. Colton T. Statistics in medicine. Boston: Little Brons and Company, 1974:189-214.

85. Daniel WW. Biostatistics: a foundation for analysis in the health sciences. New York: John Wiley & Sons, 1974:269-297.

APPENDIX A

Quick Thaw Cryoprecipitate Preparation

FFP was thawed in a 4°C circulating water bath (Neslab RTE-8, Neslab Instruments, Inc, Portsmouth, NH) without plastic overwrap. No more that three units of FFP were thawed together to allow for adequate water circulation. Units were thawed to a slushy consistency (80-90% thawed). Thawing was completed within 45-60 minutes depending on total volume of the unit. When thawing was completed, the FFP units were removed from the water bath and dried. The ice crystals were packed to the top of the unit and the units placed into a plasma expressor. A plasma transfer set was used to transfer supernatant plasma into a waste container which was placed on a digital scale (Mettler Instrument Co, Hightstown, NJ) and tared to zero. The plasma unit and waste receptacle were at the same height. Aliquots (2 ml each) were collected at specific points corresponding to 80%, 60%, 40%, and 20% of the total volume of the FFP unit as the supernatant plasma was removed. Aliquots were obtained by clamping the plasma transfer set at the specified volume to stop flow, placing the transfer tubing in the collection tube and the 2 ml aliquot collected. The transfer set was then again clamped and the tubing returned to the waste container. The units remained

in the expressor until flow of supernatant plasma stopped. Total time required for collection was approximately five minutes. The transfer tubing was then sealed and the unit of cryoprecipitate separated. Both cryoprecipitate and aliquots were returned to -70°C.

APPENDIX B

Slow Thaw Cryoprecipitate Preparation

FFP units were removed from freezer and placed in a 4°C laboratory refrigerator (Puffer Hubbard, Inc, New York, NY). Units were placed on an absorbent material without overwrap. Units were left undisturbed until completely thawed, 18-20 hours. The units were then centrifuged at 5000 g for ten minutes in a 4°C refrigerated centrifuge (Sorvall RC3B, H6000A rotor, Dupont Co, Newton, CT). The units were removed from the centrifuge, a plasma transfer set attached, and the units inverted approximately 18 inches above a digital scale (PK36 Digital Scale, Mettler Instrument Co, Highstown, NJ). The plasma transfer set was attached to a waste container on the balance and supernatant plasma allowed to flow down into the waste container. At specified points, corresponding to 80%, 60%, 40% and 20% of the total volume, 2 ml aliquots were collected by clamping the transfer tubing, and relocating it to a collection tube where the aliquot was collected. The transfer tubing was then reclamped and returned to the waste container and plasma flow was resumed. Supernatant plasma removal time was approximately 2-3 minutes. After the supernatant plasma was removed, the tubing was sealed and the cryoprecipitate unit disconnected and placed at -70°C along with the

aliquots collected until assays were performed.

APPENDIX C

Buffer Formulas

Imidazole Buffered Saline: pH 7.3 ± 0.1

314 grams Imidazole (Eastman Company, Rochester, NY)

5.85 grams sodium chloride (Sigma Chemical Company, St Louis, MO)

186 ml 0.1 N hydrochloric acid

q.s. to 1 liter with purified water

Owren's Veronal Buffer: pH 7.40 ± 0.1

5.9 grams sodium barbital

7.1 grams sodium chloride

215 ml 0.1 N hydrochloric acid

q.s. to 1 liter with purified water